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Das Österreichische Patentamt bestätigt, dass

Frank MATTNER
in A-1180 Wien, Krottenbachstraße 267,

am **17. September 2003** eine Patentanmeldung betreffend

"Verfahren zur Vorbeugung und Behandlung der Alzheimer-Erkrankung",

überreicht hat und dass die beigeheftete Beschreibung samt Zeichnungen mit der ursprünglichen, zugleich mit dieser Patentanmeldung überreichten Beschreibung samt Zeichnungen übereinstimmt.

Für diese Anmeldung wurde die innere Priorität der Anmeldung in Österreich vom 14. Jänner 2003, A 36/2003, in Anspruch genommen.

Österreichisches Patentamt
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Der Präsident:

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(56) Entgegenhaltungen, die für die Beurteilung der Patentierbarkeit in Betracht gezogen wurden:

The present invention relates to methods for preventing and treating Alzheimer's disease (AD).

Amyloid-ß peptide (Aß) plays a central role in the neuropathology of Alzheimer's disease (AD) (Roher et al 1993: "ß-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: Implications for the pathology of Alzheimer disease" PNAS 90:10836). Familial forms of the disease have been linked to mutations in the amyloid precursor protein (APP) and the presenilin genes. Disease-linked mutations in these genes result in increased production of the 42-amino acid form of the peptide (Aß42), which is the predominant form found in the amyloid plaques of Alzheimer's disease. An animal model for the disease is commercially available. The PDAPP transgenic mouse, which over-expresses mutant human APP (in which the amino acid at position 717 is F instead of V), progressively develops many of the neuropathological hallmarks of Alzheimer's disease in an age- and brain-dependent manner (Games et al 1995: "Alzheimer-type neuropathology in transgenic mice overexpressing V717F ß-amyloid precursor protein" Nature 373:523).

Vaccination studies with a "normal", not mimotope-based vaccine have already been performed. Transgenic animals were immunized with aggregated Aß42, either before the onset of AD-type neuropathologies (6 weeks) or at an older age (11 months): Immunization of young animals prevented the development of plaque formation, neuritic dystrophy and astrogliosis. Treatment of older animals markedly reduced AD-like neuropathologies. This experimental vaccination approach induced the development of antibodies against Aß42 able to cross the blood-brain barrier and attack amyloid plaques (Schenk et al 1999: "Immunization with amyloid-ß attenuates Alzheimer-disease-like pathology in the PDAPP mouse" Nature 400:173). The plaques are subsequently removed by several mechanisms, including Fc-receptor mediated phagocytosis (Bard et al 2000: "Peripherally administered antibodies against amyloid ß-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease" Nature Med 6:916). This vaccine was also able to delay memory deficits (Janus et al 2000: "Aß peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease" Nature

408:979).

A highly promising immunization therapy for AD has been in clinical trials since late 1999. Immunization is presumed to trigger the immune system to attack the plaques and clear these deposits from the affected human brain, although the precise mechanism underlying needs to be characterized in more detail.

These clinical trials were conducted by the pharmaceutical company Elan in conjunction with its corporate partner, American Home Products (therapeutic vaccine AN-1792, QS21 as adjuvant). Phase I trials were successfully completed in 2000. Phase II trials were begun late 2001 to test efficacy in a panel of patients with mild to moderate AD.

Now these phase II trials have been permanently discontinued due to neuroinflammation in several patients (Editorial 2002 "Insoluble problem?" Nature Med 8:191). The symptoms included aseptic meningoencephalitis leading to the immediate halt of these world-wide trials. In the worst case scenario, affected patients will be shown to have mounted an autoimmune response - a risk inherent in many immunotherapies. Autoimmune complications could have been anticipated given the ubiquity of APP, which of course bears antigenic determinants in common with its proteolytic product. More recently, additional studies concentrated on the nature of aggregated Aß42 immunization-induced antibodies (in humans and mice) revealing that most antibodies recognize a small domain between amino acid 4 and 10 of Aß42 (Aß4-10). The mouse antibodies were able to block Aß fibrillogenesis and disrupted pre-existing Aß fibers (McLaurin et al 2002: "Therapeutically effective antibodies against amyloid-ß peptide target amyloid-ß residues 4-10 and inhibit cytotoxicity and fibrillogenesis" Nature Med 8:1263). Of note, the human antibodies do not react with APP exposed on the surface of cells or any other non-aggregated proteolytic product of the precursor (Hock et al 2002: "Generation of antibodies specific for ß-amyloid by vaccination of patients with Alzheimer disease" Nature Med 8:1270). A clear difference was observed between human and mouse sera: In contrast to human antibodies, mouse antibodies detect monomeric, oligomeric, and fibrillar Aß. This is of importance and may be a

prerequisite for the therapeutic potency since evidence is accumulating that small oligomers of Aß, which are not recognized by human anti-Aß, are the major toxic players in the disease (Walsh et al 2002: "Naturally secreted oligomers of amyloid ß protein potently inhibit hippocampal long-term potentiation in vivo" Nature 416:535). Thus, a potential new strategy is the immunization with a vaccine containing ß-amyloid amino acids 4-10 (instead of aggregated Aß42). Despite unknown efficacy this strategy may also face autoimmune problems since patients shall be directly immunized with a (linear B cell) "self" epitope.

Despite these disappointing developments in recent AD vaccination strategies, an Aß vaccine is still regarded as the most promising way for combatting AD. However, there is an urgent need for improvements and new strategies in AD vaccination. Especially, such a vaccine should not induce autoreactive T and/or B cells.

Therefore, the present invention provides the use of a compound comprising the following amino acid sequence

$$X_1X_2X_3X_4X_5X_6,$$

wherein X_1 is an amino acid, except of C,
X_2 is an amino acid, except of C,
X_3 is an amino acid, except of C,
X_4 is an amino acid, except of C,
X_5 is an amino acid, except of C,
X_6 is an amino acid, except of C,
and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH, said compound having a binding capacity to an antibody being specific for the natural N-terminal Aß42 sequence DAEFRH, for the preparation of a vaccine for Alzheimer's disease (AD).

According to the present invention an Aß42 mimotope is used for vaccination against AD: The mimotope induces the production of antibodies against Aß42 but not against the native APP. The mimotope may be identified with a (monoclonal) antibody and (commercially available) peptide libraries (e.g. according to Reineke et al. 2002: "Identification of distinct antibody epitopes

and mimotopes from a peptide array of 5520 randomly generated sequences" J Immunol Methods 267:37). A (monoclonal) antibody is used that does not recognize APP but detects only different Aß species with amino-terminal aspartic acid (an example for such an antibody is described in Johnson-Wood et al 1997: "Amyloid precursor protein processing and Aß42 deposition in a transgenic mouse model of Alzheimer disease" PNAS 94:1550). Such an antibody has been proven to be an ideal tool to identify vaccine-suitable mimotopes in the course of the present invention. Although such monoclonal antibodies were shown to have beneficial effects in a mouse model of AD when directly administered to mice (Bard et al 2000: "Peripherally administered antibodies against amyloid ß-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease" Nature Med 6:916), these antibodies have never been proposed to be used as mimotope search tools for isolating AD vaccine compounds.

In the prior art, all efforts were concentrated on the naturally occurring Aß peptide. As mentioned above, Aß peptide vaccine clinical trials were stopped due to neuroinflammation events. Indeed, T cell epitope prediction programs (BIMAS for class I-restricted epitopes and TEPITOPE for class II-restricted epitopes) propose high score (self) epitopes within the sequence. This could imply that the neuroinflammatory events are due to autoimmune reactions which would make such a vaccine unsuitable for a general application.

In contrast to such Aß vaccines proposed by the prior art, no autoimmune reactions are expected to occur during treatment with a vaccine containing a mimotope according to the present invention, because the (monoclonal) antibody used for mimotope identification according to the present invention does not recognize APP and the mimotope sequence is different from Aß42-derived self sequences that have been used in trials so far or shall be used in future trials.

The antibody used for the mimotope identification according to the present invention detects the Aß-derived amino acid sequence DAEFRH (= original epitope) with a free amino terminal aspartic acid, thus it does not recognize native APP. The antibody may be

a monoclonal or polyclonal antibody preparation or any antibody part or derivative thereof, the only prerequisite is that the antibody molecule specifically recognises the DAEFRH epitope, i.e. that it does not bind to the natural N-terminally prolonged forms of the amyloid precursor protein, which means that the binding capacity to the DAEFRH epitope is at least 100 times, preferably at least 1000 times, more preferred at least 10^5 times, higher than to the APP molecule. The antibody may be an antibody showing the same or a higher binding capacity to the DAEFRH sequence as the antibody described by Johnson-Wood et al., 1997. Of course, also antibodies with a lower binding capacity may be used (>10 %, >50 % or >80 % of the binding capacity of the Johnson-Wood et al.antibody), although the higher binding capacity is more preferred.

The compounds according to the present invention bind to those antibodies with comparable specificity as the DAEFRH sequence.

Preferably, the compound to be used according to the present invention comprises or is consisting of a peptide, wherein
X_1 is an amino acid with a hydroxy group or a negatively charged amino acid, preferably E, Y, S or D,
X_2 is a hydrophobic amino acid or a positively charged amino acid, preferably I, L, V, K, W, R, Y, F or A,
X_3 is a negatively charged amino acid, preferably D or E,
X_4 is an aromatic amino acid or L, preferably Y, F or L,
X_5 is H, K, Y, F or R, preferably H, F or R, and
X_6 is R, I, K, Y, or G, preferably R, I or G,
especially EIDYHR, ELDYHR, EVDYHR, DIDYHR, DLDYHR, DVDYHR, DIDYRR, DLDYRR, DVDYRR, DKELRI, DWELRI, YREFRI, YAEFRG, EAEFRG, DYEFRG, ELEFRG or SFEFRG.

The compound (mimotope) according to the present invention has a preferred length of 5 to 15 amino acids. This compound may be provided in the vaccine in isolated (peptide) form or may be coupled or complexed to other molecules, such as pharmaceutical carrier substances or polypeptide, lipid or carbohydrate structures. Preferably, the mimotopes according to the present invention have a (minimum) length of between 5 and 15, 6 and 12 amino acid residues, specifically between 9 and 11. The mimotopes can,

however, be coupled (covalently or non-covalent) to unspecific linkers or carriers, especially peptide linkers or protein carriers. Furthermore, the peptide linkers or protein carriers might consist of or contain T-cell helper epitopes.

Preferably, the pharmaceutically acceptable carrier is KLH, tetanus toxoid, albumin binding protein, bovine serum albumin, a dendrimer (MAP; Biol.Chem.358:581) as well as the adjuvant substances described in Singh et al., Nat. Biotech. 17(1999), 1075-1081 (specifically those in table 1 of this document) and O'Hagan et al., Nature Reviews, Drug Discovery 2(9)(2003), 727-735 (specifically the innate immune-potentiating compounds and the delivery systems described therein), or mixtures thereof. In addition, the vaccine composition may contain aluminium hydroxyde.

A vaccine comprising the present compound (mimotope) and the pharmaceutically acceptable carrier may be administered by any suitable application mode, e.g. i.v., i.p., i.m., intranasal, oral, subcutaneous, etc. and in any suitable delivery device (O'Hagan et al., Nature Reviews, Drug Discovery 2(9)(2003), 727-735). Typically, the vaccine contains the compound according to the present invention in an amount of 0,1 ng to 10 mg, preferably 10 ng to 1 mg, especially 100 ng to 100 µg or, alternatively e.g. 100 fmole to 10 µmole, preferably 10 pmole to 1 µmole, especially 100 pmole to 100 nmole. The vaccine may also comprise typical auxiliary substances, e.g. buffers, stabilizers, etc.

According to another aspect, the present invention further relates to a method for isolating a compound binding to an antibody being specific for the natural N-terminal Aß42 sequence DAEFRH comprising the steps of

- providing a peptide compound library comprising peptides containing the following amino acid sequence

$$X_1X_2X_3X_4X_5X_6,$$

wherein X_1 is an amino acid, except of C,
X_2 is an amino acid, except of C,
X_3 is an amino acid, except of C,
X_4 is an amino acid, except of C,

X_5 is an amino acid, except of C,
X_6 is an amino acid, except of C,
and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH,
- contacting said peptide library with said antibody and
- isolating those members of the peptide library which bind to said antibody.

According to a specific embodiment of this aspect, the present invention relates to a method for isolating a compound binding to an antibody being specific for the natural N-terminal Aß42 sequence DAEFRH comprising the steps of
- providing a peptide compound library comprising peptides containing the following amino acid sequence

$$X_1X_2X_3X_4X_5X_6,$$

wherein X_1 is a natural amino acid, except of K and C,
X_2 is a natural amino acid, except of C,
X_3 is a natural amino acid, except of K and C,
X_4 is a natural amino acid, except of K and C,
X_5 is a natural amino acid, except of C,
X_6 is a natural amino acid, except of P and C,
and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH,
- contacting said peptide library with said antibody and
- isolating those members of the peptide library which bind to said antibody.

Such a method has been proven to be successful for providing Aß mimotopes according to the present invention.

Preferably, said peptides are provided in individualised form in said library, especially immobilised on a solid surface, such as e.g. possible with the MULTIPIN™ peptide technology. The library may also be provided as a peptide mixture and the antibody:peptide complexes may be isolated after antibody binding. Alternatively, the antibody may be immobilised and the peptide library (in suspension or solution) is then contacted with the immobilised antibodies.

Preferably, the screening antibodies (or the members of the peptide library) comprise a suitable marker which allows the detec-

tion or isolation of the antibody or the antibody:peptide complex when bound to a peptide of the library. Suitable marker systems (i.a. biotinylation, fluorescence, radioactivity, magnetic markers, colour developing markers, secondary antibodies) are readily available to the skilled man in the art.

The library has to be constructed to exclude the naturally occurring Aß sequence (e.g. DAEFRH), since vaccination with this sequence is clearly excluded from this invention.

A further suitable technique for isolating the epitopes according to the present invention is screening in phage-peptide libraries as e.g. described in WO 03/020750.

The present invention also relates to a vaccine against Alzheimer's Disease comprising an antigen which includes at least one peptide selected from the group EIDYHR, ELDYHR, EVDYHR, DIDYHR, DLDYHR, DVDYHR, DIDYRR, DLDYRR, DVDYRR, DKELRI, DWELRI, YREFRI, YAEFRG, EAEFRG, DYEFRG, ELEFRG or SFEFRG. These peptides are - besides the other peptides provided with the present invention specifically suited to be used for the preparation of a pharmaceutical composition, especially for AD vaccines. These sequences are purely artificial Aß-mimotopes. The peptides may - for vaccination purposes - be coupled (covalently or non-covalently) to suitable carriers and may be provided as peptide compounds or complexes together with other compounds or moieties, e.g. adjuvants, peptide or protein carriers, etc. and administered in a suitable manner (as e.g. described in O'Hagan et al., Nature Reviews, Drug Discovery 2(9)(2003), 727-735).

The invention is further described in the following examples and the drawing figures, of course without being restricted thereto.

Fig. 1 shows the individualised peptide members of library 4 used for the present screening process.

Fig. 2 shows an inhibition assay with mimotopes for DAEFRH.

Fig. 3 shows another inhibition assay with other mimotopes for DAEFRH.

EXAMPLES:

1.: Generation of monoclonal antibodies (mAb) to detect Aß42-derived peptide species with free N-terminus (free aspartic acid at the N-terminus)

Mice are vaccinated with the 6mer peptide DAEFRH (natural N-terminal Aß42 sequence) linked to the protein bovine serum albumin BSA (to make use of the hapten-carrier-effect), emulsified in CFA (first injection) and IFA (booster injections). DAEFRH-peptide-specific, antibody-producing hybridomas are detected by ELISA (DAEFRH-peptide-coated ELISA plates). Peptide SEVKMDAEFRH (natural N-terminally prolonged sequence, APP-derived, containing the Aß42-derived sequence DAEFRH) is used as negative control peptide: hybridomas recognizing the prolonged peptide are excluded because they do not distinguish between Aß42-derived peptides with free aspartic acid at the N-terminus and APP-derived peptide DAEFRH without free aspartic acid.

2.: Construction of Peptide Libraries:

The mimotopes of the present invention have been found by adapting the method of Reinke et al., 2000, by screening peptide libraries for binding to an antibody (preferably a monoclonal antibody) which is specific for Aß species with amino-terminal aspartic acid. Another method is commercially available as MULTIPIN™ peptide technology.

The Multipin™ peptide technology involves synthesizing peptides on to specially prepared polyethylene pins mounted on blocks in a format which is compatible with the standard 8 x 12 microtiter plate used for many biological assays. Both pin-bound (non-cleavable peptides which remain covalently bound to the pin) and solution phase peptides (those that have been cleaved off the pin surface) can be produced by this method. PepSets, based on the Multipin synthesis system, permit the simultaneous synthesis and screening of large numbers of peptides.

PepSets consist of blocks of 96 individually synthesized pep-

tides, two of which are carefully selected control sequences. Cleaved controls are assessed for purity by reverse phase HPLC and peptide content quantitated by amino acid analysis. Positive and negative non-cleavable controls are assessed by standard ELISA techniques.

PepSet peptides are available with a variety of chemical modifications including acetylation, biotinylation, phosphorylation and cyclization. The solution phase (cleaved) peptides are shipped as lyophilized powders.

For the production of solution phase peptides there is a choice of C-terminal endings, including acid and amide, depending on the intended peptide application. The cleavable bond is incorporated onto the pin surface, either as a preformed ester derivative of the C-terminal amino acid, or onto the "Rink" amide linker. Peptides with acid or amide end groups are then released by treating the pin-bound peptide with strong acid. Options for the scale of synthesis are a nominal 1 micromole or 5 micromole scale. Factors such as hydrophobicity and cleavage efficiency will affect peptide recovery, such that the expected yield of peptide is 0.5 to 1 micromole (around 1mg of a 15mer peptide) when the peptides are synthesized on the nominal 1 micromole scale, or a yield of 2.5 to 5 micromole for peptides synthesized on the nominal 5 micromole scale.

Non-cleavable peptides remain covalently bound to the pins and can be used to rapidly screen for peptides of interest using ELISA techniques. Such peptides are useful for the purposes of antibody epitope scanning and structure-activity relationship (SAR) studies. Removal of bound antibodies or other proteins regenerates the peptides and allows their reuse in further assays. PepSets are used for a variety of applications including the identification of peptide leads of biological interest from scanning through protein sequences, the optimization of peptide leads, and the development of new generations of analogs. Flexibility in terms of the overall strategy used in screening procedures is greatly enhanced through the use of a variety of synthesis designs which together provide a systematic method to fully characterize the lead candidate.

The comprehensive results obtained from systematic peptide sets not only identify peptides of interest, but also indicates critical residues, their replaceability and optimal peptide length. Consequently, a range of related peptides may be ranked as a result of such findings. Replacement of L-amino acids with D-amino acids and other unusual residues is a powerful approach to manipulate the structure and conformation of a peptide. This method is also a rapid way to discover new analogs with different pharmacological properties, such as antagonists and peptides with increased stability.

Starting with a known protein sequence, all sequential antibody epitopes can be mapped using the Multipin approach. Several alternative procedures for mapping sequential B-cell epitopes are now possible. These include pin-bound peptides, solution phase peptides coated directly onto microtiter plates, and biotinylated peptides captured on microtiter plates previously coated with avidin or streptavidin.

For the present examples, the antibody described in example 1 is used for screening peptide libraries, however, any antibody preparation specifically recognizing the DAEFRH-sequence, but not the naturally N-terminally prolonged sequence of the Aß molecule (e.g. MDAEFRH, KMDAEFRH, SEVKMDAEFRH or the complete amyloid (precursor) protein, APP), such as e.g. described by Johnson-Wood et al., 1997.

Four libraries have been constructed for this purpose:

2.1.: Library 1: This 6mer library contains peptides with the following sequences (amino acid positions 1 to 6):

Position 1: all natural aa except of D, K, and C (17 possibilities)
Position 2: all natural aa except of A, K, and C (17 possibilities)
Position 3: all natural aa except of E, K, and C (17 possibilities)
Position 4: all natural aa except of F, K, and C (17 possibili-

ties)
Position 5: all natural aa except of R, K, and C (17 possibilities)
Position 6: all natural aa except of H, K, C, and P (16 possibilities)

Library 1 is a mixture of hexapeptides. Theoretically, all possible peptides containing 17 different amino acids (see below) are included. The mixture does not contain any lysine and cysteine residue. Furthermore, the mixture does not contain:
aspartic acid at the specific position 1,
alanine at the specific position 2,
glutamic acid at the specific position 3,
phenylalanine at the specific position 4,
arginine at the specific position 5, and
histidine at the specific position 6.

The synthesis is performed on an Applied Biosystems 431A-Synthesizer following the FastMoc protocol, with a synthesis scale of 0.25 mmol.

The synthesis starts with weighing 1 mmol of all desired amino acids (amino groups and side chains protected). Then, a mixture of Asn, Gln, Gly, Ile, Leu, Met, Pro, Ser, Thr, Trp, Tyr, Val was produced. Position-specific, the following amino acids are added:
Ala, Glu, Phe, Arg, His (position/mixture 1),
Asp, Glu, Phe, Arg, His (position/mixture 2),
Asp, Ala, Phe, Arg, His (position/mixture 3),
Asp, Ala, Glu, Arg, His (position/mixture 4),
Asp, Ala, Glu, Phe, His (position/mixture 5), and
Asp, Ala, Glu, Phe, Arg (position/mixture 6, without Pro).

Mixture 6 was used to load the resin (2-chloro-tritylchloride resin, 1.49 mmol/g, Alexis Germany):
1 mmol amino acid residue mixture 6
611 mg resin (= 0.91 mmol reactive groups)
15 ml dichloromethane
5.5 equivalent = 5 mmol diisopropylethylamine (871 µl).

The mixture is shaken in a flask for 1 h. Then, 1 ml methanol is added and the mixture is shaken for an additional 10 min. The loaded resin is extracted via a frit and washed twice with dimethylformamide, dichlormethane, isopropanol, methanol, and ether (30 ml of each). The drying is performed overnight in a high vacuum. The weigh-out quantity is 737 mg.

An aliquot of 5.66 mg is treated for 30 min with 1 ml of 20% piperidine in DMF to define the density of the resin. Then, the mixture is centrifuged. The free Fmoc protective group is photometrically measured in the supernatant (301 nm, coefficient of extinction = 7800 M (e-1)). Accordingly, the density of the resin is 0.49 mmol/g.

All following steps are performed at the synthesizer, using the other mixtures (put in 5 different cartridges). 515 mg of loaded resin are used (corresponding to 0.25 mmol: amino acid mixtures are used in 4-times excess). The N-terminal Fmoc protective group is cleaved at the end of the synthesis. After washing with ethanol and drying overnight, cleavage of the peptides from the resin is accomplished by TFA/H_2O (95:5, v:v). The TFA solution is concentrated in a Speed Vac to 1/5 volume and precipitated and washed in diethylether and lyophilized.

The 6mer peptides EIDYHR, ELDYHR, and EVDYHR are examples for mimotopes that can be detected by the monoclonal antibody produced according to example 1. above.

2.2.: Library 2: This 6mer library contains peptides with the following sequences (amino acid positions 1 to 6):

Position 1: D (fixed)
Position 2: all natural aa except of A, K, and C (17 possibilities)
Position 3: all natural aa except of E, K, and C (17 possibilities)
Position 4: all natural aa except of F, K, and C (17 possibilities)
Position 5: all natural aa except of R, K, and C (17 possibili-

Position 6: all natural aa except of H, K, C, and P (16 possibilities).

Peptide library 2 was constructed according to the method described above (under 2.1) for library 1.

The 6mer peptides DIDYHR, DLDYHR, and DVDYHR are examples for mimotopes that can be detected by the monoclonal antibody produced according to 1. above.

2.3.: Library 3: A third peptide library is used in an additional approach to define mimotope sequences. This library contains the original sequence, and allows the detection of mimotopes more closely related to the original epitope.

This 6mer library contains peptides with the following sequences (amino acid positions 1 to 6):

Position 1: all natural aa except of K, and C (18 possibilities)
Position 2: all natural aa except of K, and C (18 possibilities)
Position 3: all natural aa except of K, and C (18 possibilities)
Position 4: all natural aa except of K, and C (18 possibilities)
Position 5: all natural aa except of K, and C (18 possibilities)
Position 6: all natural aa except of K, C, and P (17 possibilities).

Peptide library 3 was constructed according to the method described above (under 2.1) for library 1.

The 6mer peptides DIDYRR, DLDYRR, and DVDYRR are examples for mimotopes that can be detected by the monoclonal antibody produced according to 1. above (D in position 1 and R in position 5 are identical with the original epitope).

2.4.: Library 4: This peptide library 4 consists of 5 x 18 = 90 peptides, is commercially available from Mimotopes Ltd. (Paris, France; see manufacturer's guidelines) and is designed according to the natural N-terminal Aß42 sequence DAEFRH.

Position 1: D (fixed)

Position 2: all natural amino acids except of K and C (18 different peptides)
Position 3: all natural amino acids except of K and C (18 different peptides)
Position 4: all natural amino acids except of K and C (18 different peptides)
Position 5: all natural amino acids except of K and C (18 different peptides)
Position 6: all natural amino acids except of K and C (18 different peptides).

The individualised peptide members of library 4 are depicted in fig. 1. Peptides no. 1, 24, 48, 56 and 80 have the original sequence of the Aß42 N-terminal sequence. All other peptides are candidate peptides which are tested with respect to their binding capacity to a DAEFRH-binding antibody.

2.5.: ELISA with peptide libraries:

As mentioned above, peptide libraries 1, 2, and 3 are generated with an Applied Biosystems 431A peptide synthesizer following classical Fmoc-chemistry. The commercially available peptide library 4 is generated according to the manufacturer's description (see above and under www.mimotopes.com). The 90 peptides are C-terminally linked to a pin.

The ELISA with each of the peptide libraries have been carried out following standard protocols:

The peptide library is dissolved in 100% DMSO (final concentration 10 mg/ml).
The peptide solution is further diluted in PBS.
The peptide mixture is coated overnight (4 °C) onto ELISA plates (Nunc Maxisorp, Germany), starting with 500 µg/well, and titrated to 100 ng/well.
The plates are washed 3x times with PBS/Tween 20 (0.1% v/v).
The plates are blocked with PBS/BSA (2 h at room temperature).
The plates are washed 3x times with PBS/Tween.
The plates are incubated with biotinylated DAEFRH-specific mAb (10 µg/ml in PBS) for 4 h at room temperature.

The plates are washed 3x times with PBS/Tween.
The plates are incubated with streptavidin-horseradish-peroxidase (30 min at room temperature).
The plates are washed 5x times with PBS/Tween.
The plates are incubated with ABTS + H_2O_2 (0.1 % w/v; 10 to 45 min) and the reaction is stopped with citric acid followed by photometric evaluation (wavelength 405 nm).

3.: Verification of Mimotopes by Inhibition Assay

3.1. Additional library

In addition to the 4 libraries described above (see 2.1., 2.2., 2.3., and 2.4.) a fifth library is used to define mimotope sequences. This 6mer library is commercially available at EMC microcollections (Tübingen Germany) and contains 114 different hexapeptide mixtures, one position per mixture is defined by one of all natural aa except of C (19 possibilities), the remaining 5 positions are variable:

Mixtures 01 to 06 (one position fixed, alanine A, remaining 5 variable, X):

Mixture 01: AXXXXX
Mixture 02: XAXXXX
Mixture 03: XXAXXX
Mixture 04: XXXAXX
Mixture 05: XXXXAX
Mixture 06: XXXXXA

Mixtures 07 to 12 (one position fixed, arginine R, remaining 5 variable, X):

Mixture 07: RXXXXX
Mixture 08: XRXXXX
Mixture 09: XXRXXX
Mixture 10: XXXRXX
Mixture 11: XXXXRX
Mixture 12: XXXXXR

Accordingly, mixtures 13 to 114 are designed using all natural aa except of C.

3.2. Inhibition assay

Figures 2 and 3 describe the results of inhibition assays performed with mimotope peptides included in and obtained from the 5 libraries (as described). The mimotope peptides compete with the orginal epitope for recognition by the monoclonal antibody. Original epitope and mimotope peptides contain an additional C at the C-terminus for coupling to a protein carrier (if desired).

The following peptides are used:

Peptide 1737 DAEFRH
Peptide 3001 DKELRI
Peptide 3002 DWELRI
Peptide 3003 YREFFI
Peptide 3004 YREFRI
Peptide 3005 YAEFRG
Peptide 3006 EAEFRG
Peptide 3007 DYEFRG
Peptide 3008 ELEFRG
Peptide 3009 SFEFRG
Peptide 3010 DISFRG
Peptide 3011 DIGWRG

Procedure:
ELISA plates (Nunc Maxisorp) are coated with the original peptide epitope DAEFRH (C-terminally prolonged with C and coupled to bovine serum albumin BSA) at a concentration of 0.1 µg/ml peptide-BSA (100µl/well, 12h, 4°C). After blocking with PBS/BSA 1% (200µl/well, 12h, 4°C), the plates are washed 3x times with PBS/Tween. Then, biotinylated monoclonal antibody (1:2000, 50µl/well) and peptides (50µl/well) at 50, 5, 0.5, 0.05, 0.005, and 0.0005 µg/ml are added for 20 min. at 37°C. The plates are washed 3x times with PBS/Tween and are incubated with horseradish peroxidase (HRP)-labeled streptavidin (100µl/well, 30 min, RT). The plates are washed 5x times with PBS/Tween and are incu-

bated with ABTS + H_2O_2 (0.1% w/v, 10 to 45 min) and the reaction is stopped with citric acid followed by photometric evaluation (wavelength 405 nm).

As expected and seen in Fig.2, peptide 1737 DAEFRH can compete with BSA-coupled, plate-bound peptide DAEFRH and thus inhibits recognition by the monoclonal antibody. Furthermore, it is shown that peptide 3003 is not able to inhibit binding of the monoclonal antibody to the original epitope. In contrast, peptides 3001, 3002, 3004, 3005, 3006, and 3007 (to a different extent) block epitope recognition. Whereas peptide 3004 is only inhibitory at a high concentration (50 µg/ml), peptides 3001, 3006, and 3007 are strongly inhibitory with an IC_{50} of less than 0.5 µg/ml. Peptides 3002 and 3005 are "intermediate" inhibitors with an IC_{50} of more than 0.5 µg/ml.

As expected and seen in Fig.3, peptide 1737 DAEFRH can successfully compete with BSA-coupled, plate-bound peptide DAEFRH for monoclonal antibody recognition in an additionally performed, independent experiment. Furthermore, it is shown that peptides 3010 and 3011 are not inhibitory at the concentrations tested, whereas peptides 3008 and 3009 are (relatively) weak inhibitors with an IC_{50} of less than 5 µg/ml.

Table 1 briefly summarizes the inhibitory capacity of mimotopes included in and obtained from libraries (as described):

Table 1: Inhibitory capacity of mimotopes:

Peptide 3001	DKELRI	strong
Peptide 3002	DWELRI	intermediate
Peptide 3003	YREFFI	none
Peptide 3004	YREFRI	weak
Peptide 3005	YAEFRG	intermediate
Peptide 3006	EAEFRG	strong
Peptide 3007	DYEFRG	strong
Peptide 3008	ELEFRG	weak
Peptide 3009	SFEFRG	weak
Peptide 3010	DISFRG	none
Peptide 3011	DIGWRG	none

Claims:

1. Use of a compound comprising the following amino acid sequence

$$X_1X_2X_3X_4X_5X_6,$$

wherein X_1 is an amino acid, except of C,
X_2 is an amino acid, except of C,
X_3 is an amino acid, except of C,
X_4 is an amino acid, except of C,
X_5 is an amino acid, except of C,
X_6 is an amino acid, except of C,
and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH, said compound having a binding capacity to an antibody being specific for the natural N-terminal Aß42 sequence DAEFRH, for the preparation of a vaccine for Alzheimer's disease (AD).

2. Use according to claim 1 characterised in that said compoun comprises or is consisting of a peptide, wherein
X_1 is an amino acid with a hydroxy group or a negatively charged amino acid, preferably E, Y, S or D,
X_2 is a hydrophobic amino acid or a positively charged amino acid, preferably I, L, V, K, W, R, Y, F or A,
X_3 is a negatively charged amino acid, preferably D or E,
X_4 is an aromatic amino acid or L, preferably Y, F or L,
X_5 is H, K, Y, F or R, preferably H, F or R, and
X_6 is R, I, K, Y, or G, preferably R, I or G,
especially EIDYHR, ELDYHR, EVDYHR, DIDYHR, DLDYHR, DVDYHR, DIDYRR, DLDYRR, DVDYRR, DKELRI, DWELRI, YREFFI, YREFRI, YAEFRG, EAEFRG, DYEFRG, ELEFRG or SFEFRG.

3. Use according to claim 1 or 2 characterised in that the com pound is a polypeptide comprising 5 to 15 amino acid residues.

4. Use according to any one of claims 1 to 3 characterised is that the compound is coupled to a pharmaceutically acceptable carrier, preferably KLH, and optionally aluminium hydroxide.

5. Use according to any one of claims 1 to 4 characterised in that it contains the compound in an amount of 0,1 ng to 10 mg,

preferably 10 ng to 1 mg, especially 100 ng to 100 µg.

6. Method for isolating a compound binding to an antibody being specific for the natural N-terminal Aß42 sequence DAEFRH comprising the steps of

- providing a peptide compound library comprising peptides containing the following amino acid sequence

$$X_1X_2X_3X_4X_5X_6,$$

wherein X_1 is an amino acid, except of C,
X_2 is an amino acid, except of C,
X_3 is an amino acid, except of C,
X_4 is an amino acid, except of C,
X_5 is an amino acid, except of C,
X_6 is an amino acid, except of C,
and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH,

- contacting said peptide library with said antibody and
- isolating those members of the peptide library which bind to said antibody.

7. Method according to claim 6, characterised in that said peptides are provided in individualised form in said library, especially immobilised on a solid surface.

8. Method according to claim 6 or 7, characterised in that said antibody comprises a suitable marker which allows its detection or isolation when bound to a peptide of the library.

9. Vaccine against Alzheimer's Disease comprising an antigen which includes at least one peptide selected from the group EIDYHR, ELDYHR, EVDYHR, DIDYHR, DLDYHR, DVDYHR, DIDYRR, DLDYRR, DVDYRR, DKELRI, DWELRI, YREFRI, YAEFRG, EAEFRG, DYEFRG, ELEFRG or SFEFRG.

Summary:

The invention relates to the use of a compound comprising the following amino acid sequence

$$X_1X_2X_3X_4X_5X_6,$$

wherein X_1 is an amino acid, except of C,
X_2 is an amino acid, except of C,
X_3 is an amino acid, except of C,
X_4 is an amino acid, except of C,
X_5 is an amino acid, except of C,
X_6 is an amino acid, except of C,
and wherein $X_1X_2X_3X_4X_5X_6$ is not DAEFRH, said compound having a binding capacity to an antibody being specific for the natural N-terminal Aß42 sequence DAEFRH, for the preparation of a vaccine for Alzheimer's disease.

Fig.1A

1	alanine	ala	A
2	arginine	arg	R
3	asparagine	asn	N
4	aspartic acid	asp	D
5	cysteine	cys	C
6	glutamine	gln	Q
7	glutamic acid	glu	E
8	glycine	gly	G
9	histidine	his	H
10	isoleucine	ile	I
11	leucine	leu	L
12	lysine	lys	K
13	methionine	met	M
14	phenylalanine	phe	F
15	proline	pro	P
16	serine	ser	S
17	threonine	thr	T
18	tryptophan	trp	W
19	tyrosine	tyr	Y
20	valine	val	V

Fig.1B

No.	D	A	E	F	R	H	
1	D	A	E	F	R	H	positive control
2	D	R	E	F	R	H	
3	D	N	E	F	R	H	
4	D	D	E	F	R	H	
5	D	Q	E	F	R	H	
6	D	E	E	F	R	H	
7	D	G	E	F	R	H	
8	D	H	E	F	R	H	
9	D	I	E	F	R	H	
10	D	L	E	F	R	H	
11	D	M	E	F	R	H	
12	D	F	E	F	R	H	
13	D	P	E	F	R	H	
14	D	S	E	F	R	H	
15	D	T	E	F	R	H	
16	D	W	E	F	R	H	
17	D	Y	E	F	R	H	
18	D	V	E	F	R	H	
19	D	A	A	F	R	H	
20	D	A	R	F	R	H	
21	D	A	N	F	R	H	
22	D	A	D	F	R	H	
23	D	A	Q	F	R	H	
24	D	A	E	F	R	H	positive control
25	D	A	G	F	R	H	
26	D	A	H	F	R	H	
27	D	A	I	F	R	H	
28	D	A	L	F	R	H	
29	D	A	M	F	R	H	
30	D	A	F	F	R	H	
31	D	A	P	F	R	H	
32	D	A	S	F	R	H	
33	D	A	T	F	R	H	
34	D	A	W	F	R	H	
35	D	A	Y	F	R	H	
36	D	A	V	F	R	H	
37	D	A	E	A	R	H	
38	D	A	E	R	R	H	
39	D	A	E	N	R	H	
40	D	A	E	D	R	H	
41	D	A	E	Q	R	H	
42	D	A	E	E	R	H	
43	D	A	E	G	R	H	
44	D	A	E	H	R	H	
45	D	A	E	I	R	H	
46	D	A	E	L	R	H	
47	D	A	E	M	R	H	positive control
48	D	A	E	F	R	H	
49	D	A	E	P	R	H	
50	D	A	E	S	R	H	

Fig.1C

51	D	A	E	T	R	H	
52	D	A	E	W	R	H	
53	D	A	E	Y	R	H	
54	D	A	E	V	R	H	
55	D	A	E	F	A	H	
56	D	A	E	F	R	H	positive control
57	D	A	E	F	N	H	
58	D	A	E	F	D	H	
59	D	A	E	F	Q	H	
60	D	A	E	F	E	H	
61	D	A	E	F	G	H	
62	D	A	E	F	H	H	
63	D	A	E	F	I	H	
64	D	A	E	F	L	H	
65	D	A	E	F	M	H	
66	D	A	E	F	F	H	
67	D	A	E	F	P	H	
68	D	A	E	F	S	H	
69	D	A	E	F	T	H	
70	D	A	E	F	W	H	
71	D	A	E	F	Y	H	
72	D	A	E	F	V	H	
73	D	A	E	F	R	A	
74	D	A	E	F	R	R	
75	D	A	E	F	R	N	
76	D	A	E	F	R	D	
77	D	A	E	F	R	Q	
78	D	A	E	F	R	E	
79	D	A	E	F	R	G	
80	D	A	E	F	R	H	positive control
81	D	A	E	F	R	I	
82	D	A	E	F	R	L	
83	D	A	E	F	R	M	
84	D	A	E	F	R	F	
85	D	A	E	F	R	P	
86	D	A	E	F	R	S	
87	D	A	E	F	R	T	
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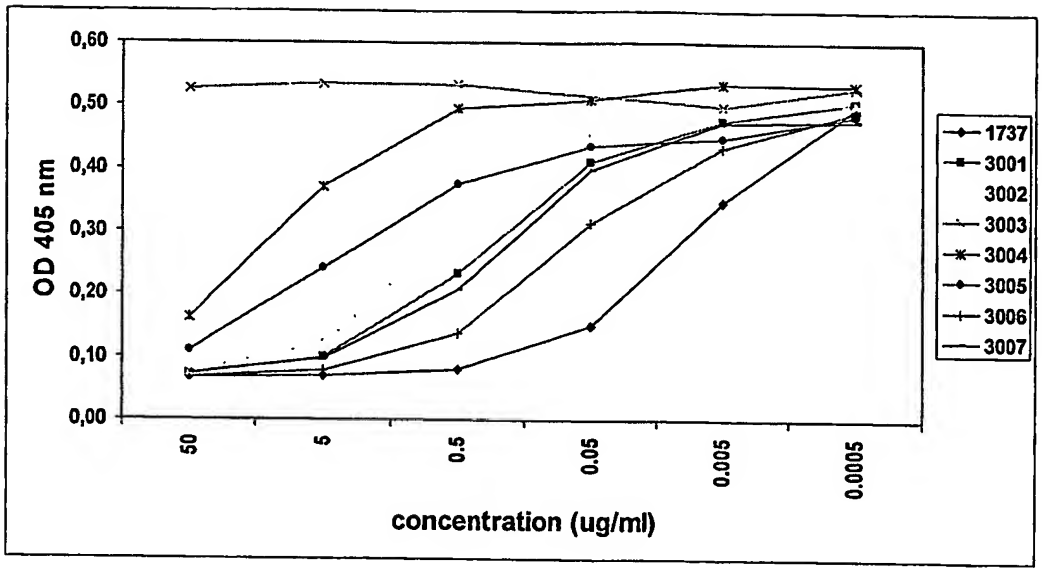
90	D	A	E	F	R	V	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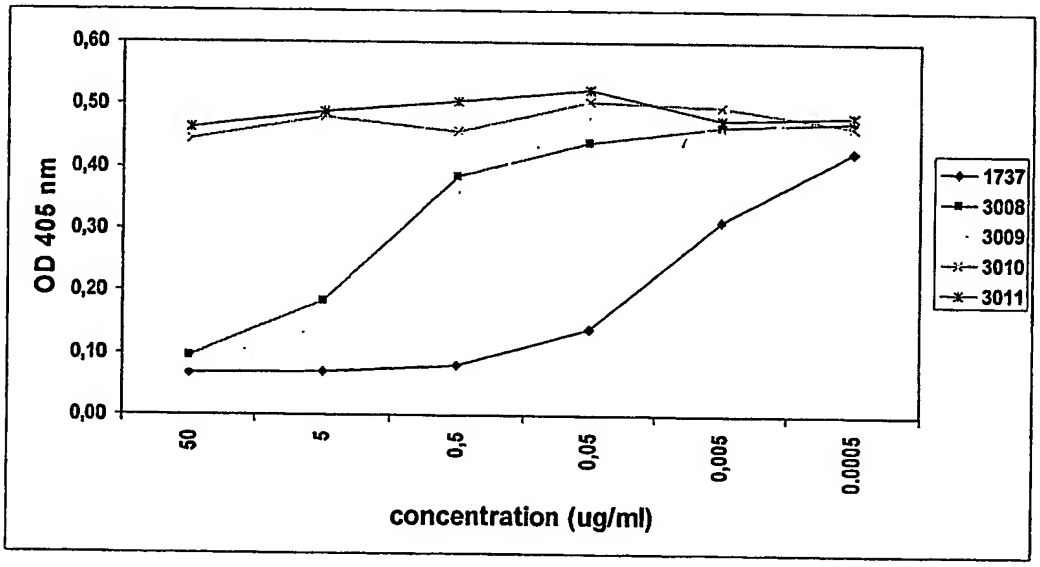
concentration (ug/ml)
1737
3001
3002
3003
3004
3005
3006
3007

Fig.2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concentration (ug/ml)
1737
3008
3009
3010
3011

Fig.3